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16. ABSTRACT

Temperature effects on the hatching success of eggs of the white bass (Morone chrysops) were determined by exposing sample lots of recently fertilized eggs to 10 constant temperatures, 6 through 30°C. Different lots of eggs were exposed to test temperatures before gastrulation, and after closure of the blastopore in the embryo. The percentages of normal larvae hatched were not significantly ($P > 0.05$) impaired in eggs exposed before gastrulation at temperatures from 18 through 26°C, nor in those first exposed after closure of the blastopore at temperatures from 14 through 26°C. Eggs incubated in 14°C hatched 4.5 days after fertilization, and those incubated at 26°C required only 1 day. The 24-h TL_{50} for white bass larvae exposed within 24 h of hatching and acclimated to 14, 18, 20, and 26°C was between 30 and 32°C, and was not altered by the acclimation temperatures tested.

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Effects of Temperature on Hatching Success and Survival of Larvae in the White Bass

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ABSTRACT: Temperature effects on the hatching success of eggs of the white bass *(Morone chrysops)* were determined by exposing sample lots of recently fertilized eggs to 10 constant temperatures, 6 through 30°C. Different lots of eggs were exposed to test temperatures before gastrulation, and after closure of the blastopore in the embryo. The percentages of normal larvae hatched were not significantly ($P > 0.05$) impaired in eggs exposed before gastrulation at temperatures from 18 through 26°C, nor in those first exposed after closure of the blastopore at temperatures from 14 through 26°C. Eggs incubated at 14°C hatched 4.5 days after fertilization, and those incubated at 26°C required only 1 day. The 24-h TL_{50} for white bass larvae exposed within 24 h of hatching and acclimated to 14, 18, 20, and 26°C was between 30 and 32°C, and was not altered by the acclimation temperatures tested.

As man's capacity to influence the environment has increased, his need to know the effects of such perturbations on various life forms has also increased — both to use such capacities beneficially, and to hold such changes within acceptable limits. The object of the present study[1] was to obtain information on the effects of a range of temperatures on three early life stages of the white bass *(Morone chrysops)*: eggs containing embryos before gastrulation, eggs containing embryos after closure of the blastopore, and newly hatched larvae (less than 24 h old). These stages are believed to be of particular importance, since they are commonly considered critical in establishing the year-class strength of a species (Kramer 1969). These early, immobile stages are particularly vulnerable to environmental perturbations, such as heated discharges, because they cannot avoid areas of elevated temperatures. Thermally induced responses of fish differ not only between species but within a species from one life stage to another (McCormick 1976; Nat. Acad. Sci. 1973). The findings of such studies should provide a scientific basis for environmental regulatory agencies to use in establishing water quality standards where this species and these life stages are involved, and criteria for fish-cultural agencies to use in selecting incubation temperatures for maximum success.

The determination of thermal requirements for the well-being of white bass is of particular interest, not only because of the importance of this species as both a sport and a commercial fish (Howell 1945; Ruelle 1971; Scott and Crossman 1973; Great Lakes Commission 1974) but also because it inhabits large bodies of fresh water where perturbation of natural thermal regimes is most likely to occur as a result of steam electric generation. Previous studies of thermal requirements of this species have been limited or were too general to identify upper and lower thermal limits for hatching and larval survival (Yellayi and Kilambi 1969).

Materials and Methods

Sexually mature white bass collected on 5-6 June 1974 from spawning populations in Sturgeon Lake, a backwater area of the Mississippi River near Red Wing, Minnesota, were brought to the Environmental Research Laboratory at Duluth, Minnesota. Water temperatures in the collection area during and immediately before netting ranged from about 16 to 20°C. The fish were held at 16°C until 8 June, when the temperature was raised to 19°C. Each of the females to be used as the egg source was then injected intraperitoneally with 1 mg of dried triturated carp pituitary in 1 ml of Cortland physiological saline solution (Sneed and Clemens 1960). Viable eggs were obtained from only one female on 9 June and fertilized with the milt from two males, using the dry method (Davis 1956). The resulting embryos were used as the test specimens. Duplicate lots of 50 embryos each were exposed to test temperatures. (After water hardening, the adhesive choreons were teased free of the spawn-taking pan for counting into test lots.) Exposures were initiated at two times to determine whether embryonic stage at first exposure to test temperatures had any effect on thermal tolerance

[1] Essentially this same material was published in the EPA Ecological Research Series, EPA-600/3-77-075, "Effects of temperature on hatching success and survival of larvae in the white bass" (1977). An edited version of the original report is published here to ensure wider dissemination of the data.

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(Kelly 1968; Frank 1974). Initial exposures to test temperatures (6 through 28°C) were completed by the 32-cell stage, Oppenheimer stage 7 (Rugh 1962), before gastrulation. This set of exposures was designated group I. Embryos in group II were held as a group at 18°C for later exposure after closure of the blastopore, between Oppenheimer stages 17 and 18. Infertile eggs and dead embryos were removed from group II before sampling and distribution to test temperatures (6 through 30°C).

The upper lethal temperature range necessary to achieve 100% mortality in group I was not reached; therefore, the range of tested temperatures was expanded for group II. The expanded thermal range was to insure sufficiently high temperatures to exceed the upper lethal limit, and to allow for possibly greater tolerance of more advanced embryos.

Tests lots were inspected periodically to determine stage of development and to remove, count, and record dead eggs and embryos. As the hatching date approached, the embryos were inspected daily. The newly hatched larvae were removed, categorized, and counted as dead, deformed, or "normal." All larvae not obviously deformed when examined under 30× magnification were considered to be normal. Time to hatching, when the first 50% or more of any test lot had hatched, was assessed to the nearest 12 h.

The embryos were exposed to test temperatures in 4- × 4-cm glass jars with open tops and 0.23-mm-mesh stainless steel screen bottoms. The jars were supported on 13-mm glass legs that held the screens off the bottoms of the water baths to allow free circulation of water past the embryos. The water baths were 10 × 40 × 10 cm deep and received a continuous mean flow of 215 ml/min (range, 200 to 250 ml/min) of Lake Superior water at constant temperatures. At the drain end of each water bath a self-starting siphon provided an oscillating water depth that provided a flow-through velocity of 4.5-5.9 cm/min during draw-down and 0.4 - 0.5 cm/min during filling. The ratio of draw-down to filling time was about 1:9.

Water temperatures were regulated by a thermostatically controlled immersion heater system and monitored by a Honeywell multipoint recording telethermometer. The recorder values were verified daily by thermometer readings in each water bath. Verification thermometers were standardized to within ± 0.1°C of a calibration thermometer certified by the American Society for Testing and Materials. Water drawn directly from Lake Superior had the following chemical characteristics: total hardness, 43 - 47 mg/l; total alkalinity, 42 - 43 mg/l; and pH 7.1 - 7.3. Dissolved oxygen concentrations within the exposure chambers were 7 mg/l or greater, and the upper limit was controlled to 104% saturation or lower by air-bubble stripping before delivery to the exposure water baths. Other chemical characteristics of the test water were essentially unchanged from those reported by Biesinger and Christensen (1972). Lighting was by Duro-Test Vita-Lite fluorescent lamps with the photoperiod normal for the time of year at Duluth, Minnesota.

The results are reported as the percentage of eggs producing normal larvae. Dead and deformed larvae were not considered for analysis because they would not (in the case of dead larvae) or were not believed likely to (in the case of deformed larvae) contribute to year-class recruitment (Volodin 1960; Kokurewicz 1969). The percentage normal hatch data were converted to arcsin percentages and analyzed by one-way analysis of variance. Tukey's multiple range tests were used to identify treatment effects statistically significant at the 95% probability level (Steel and Torrie 1960).

The upper thermal 24-h TL_{50} values of newly hatched larvae from embryos incubated at 14, 18, 20, and 26°C were determined. The upper tolerance limits were determined in the same water-bath system used for the embryo studies. Single lots of 10 larvae each were tested at 26, 28, 30, 32, and 34°C. The data were analyzed by probit analysis (Finney 1964) to derive the TL_{50} values and the 95% confidence limits.

Results and Discussion

Among group I embryos the maximum mean percentage of normal larvae (59%) was from embryos incubated at 26°C (Table 1). Those exposed at 18 through 26°C produced normal larvae at percentages not significantly less than those at 26°C (Table 1). Normal larvae were hatched but in significantly reduced numbers at 14 and 28°C.

Among group II embryos the maximum mean percentage of normal larvae (87%) was produced at 18°C. The percentages of normal larvae produced at temperatures from 14 and 26°C were not significantly less than those at 18°C (Table 1). Normal larvae were hatched but in significantly reduced numbers at 10 and 28°C.

Rates of development increased directly with temperature. From fertilization to hatching, about 4.5 days were required at 14°C, but only 1 day was required at 26°C. Thermal tolerance of newly hatched larvae did not change with acclimation. Larvae acclimated at 14, 18, 20, and 26°C all had 24-h TL_{50}'s near 31°C; overlapping 95% confidence limits ranged from 28.9°C to nearly 33.6°C (Table 2).

Hatching success in group I was uninhibited from 18 to 26°C. This range extends well beyond the optimum temperature limits of 15.6 through 16.7°C previously reported by Yellayi and Kilambi (1969). Their suggested upper lethal limit of 20.0°C was also considered to be too restricted, since normal larvae in this study were produced through 28°C, although success at the upper temperature was reduced. The lower lethal limit of 12.8°C reported by Yellayi and Kilambi (1969) seems a reasonable estimate of expected 100% mortality, based on the downward trend between 18 and 10°C in the data reported here.

Table 1. Percentage normal hatch of white bass embryos exposed to constant temperatures. Mean percentage hatched followed by asterisks are not significantly different ($P < 0.05$) from the maximum mean percentage hatch.[a]

Nominal temperature (°C)	Group I (before gastrulation)				Group II (after blastopore closure)			
	Temperature (°C, mean and range)	Replicate A	Replicate B	Mean of A and B	Temperature (°C, mean and range)	Replicate A	Replicate B	Mean of A and B
6	5.8 (5.4-6.5)	0	0	0	6.0 (5.4-6.5)	0	0	0
10	9.8[b] (9.0-11.0)	0	0	0	10.0 (9.2-11.0)	34	22	28
14	14.2 (13.3-14.8)	28	24	26	14.2 (13.3-14.8)	76	72	74*
18	18.0 (17.8-18.2)	34	30	32*	18.0 (17.8-18.2)	84	90	87*
20	20.1 (19.8-20.5)	38	48	43*	20.1 (19.8-20.5)	78	74	76*
22	21.8 (21.4-22.0)	66	50	58*	21.8 (21.4-22.0)	66	78	72*
24	23.7 (23.3-23.8)	30	32	31*	23.6 (23.3-23.8)	78	84	81*
26	25.9 (25.7-26.1)	56	62	59*	26.1 (25.7-26.3)	58[c]	74	66*
28	28.0 (27.7-28.1)	28	18	23	27.9 (27.5-28.2)	50	58	54
30	—	—	—	—	30.2 (30.0-30.5)	0	0	0

[a] When in the transformed form for statistical testing. Tukey's significant ($P = 0.05$) difference for the before-gastrulation results was 16.4% ($P = 0.05$), for the after-closure-of-the-blastopore data it was 20.2% ($P = 0.05$). All data here are presented as before normalization and transformation.

[b] Exposed during the first 14 h to mean of 9.2°C, thereafter to 10.0°C until hatching.

[c] Probably would have been higher, but the container holding embryos was tipped during part of the day of hatching, so some swimming larvae could have escaped.

The overall greater percentages of successful hatching at temperatures between 18 and 26°C among group II embryos than among group I embryos were not considered a function of the stage of development at first exposure. They are rather the result of the removal of infertile eggs and dead embryos carried out before counting out the test lots of the more advanced embryos. Thus, group II samples included a larger number of fertilized eggs and a greater percentage hatch should have been expected.

Not only was hatching success affected by temperature, but so also was incubation time from fertilization to hatching. Incubation time was shorter at higher temperatures (only 1 day or less at 28 and 26°C) and longer at lower temperatures (about 4.5 days at 14°C). At intermediate temperatures (24, 22, 20, and 18°C), incubation times were relatively lengthened at progressively lower temperatures (1.5, 2, 2, and 3.5 days, respectively).

The 24-h TL_{50} values determined for 1-day-old white bass were similar over the range of acclimations from 14 to 26°C. Although acclimation has generally been accepted as a mechanism by which fish are able to adjust their thermal tolerance, the early larval stage studied in these tests is apparently unable to make the necessary physiological changes. This result was consis nt with responses among 1-day-old brook trout *(Salvelinus fontinalis)* reported by McCormick et al. (1972). McCauley (1963) suggested that the capacity for an organism to adjust to temperature changes probably requires functional organ systems not present in early embryonic forms.

The life stage of an organism is important in determining its responses to environmental temperatures. In the present study, this was illustrated by the greater temperature tolerance range when embryos were first exposed after closure of the blastopore rather than before. The results also corroborate Badenhuizen's (1968) finding with embryos of the largemouth bass *(Micropterus salmoides)*. He also found that cold tolerance increased more with increased age of the embryo than does heat tolerance. Thermal requirements determined in the present study correlated well with naturally occurring environmental requirements. The lower limits for

Table 2. The 24-h LC_{50}'s of newly hatched larval white bass.[a]

Acclimation temperature[b] and (indented) test temperature (°C)	Mortality (%)	TL_{50} and (in parentheses) 95% confidence level
14°C		
34.2	100	31.7 (30.1-33.0)
32.3	40	
30.1	100[c]	
28.2	10	
26.2	0	
18°C		
34.4	100	30.8 (_____)[d]
32.3	100	
30.3	10	
28.0	0	
26.4	0	
20°C		
34.4	100	32.0 (31.1-33.0)
32.3	40	
30.3	20	
28.0	0	
26.4	0	
26°C		
34.2	100	30.6 (29.0-31.9)
32.3	70	
30.3	20	
28.0	30	
26.4	90[c]	
14-26°C		
34.4	100	31.3 (28.9-33.6)
32.3	62	
30.3	17	
28.0	10	
26.3	0	

[a] N = 10, all test groups.
[b] Acclimation temperature is also the incubation temperature.
[c] Points arbitrarily considered aberrant, listed for completeness of data but not used in analysis.
[d] Two values between 0 and 100% effect required for Finney's (1964) method; thus a graphic method with logarithmic probability paper was used to find this TL_{50}, and no 95% confidence level was derived.

successful incubation (between 10 and 14°C) are close to the lower thermal threshold for initiating spawning (11 through 16°C) reported by Riggs (1955), Webb and Moss (1967), Horrall (1961), and Vincent (1968), suggesting that natural stabilizing selection (Barash 1977) influences the temperature at which spawning occurs.

These same observers report spawning in natural waters at temperatures up to 23.9°C, which is somewhat lower than the upper limit of 26°C found in the present study to permit uninhibited hatching success; however, allowing for seasonally rising temperature trends during the time before hatching, this difference is not considered unreasonable. If the possibilities are accepted that field observations may misinterpret ripe-fish activity for spawning and that a few late spawners may go unobserved after the peak spawning masses leave the spawning area, field observations of suitable spawning and incubation temperatures and laboratory-derived values may be construed as reasonable approximations of each other.

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